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Irresistible curves

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The DivIVA protein helps to organize cell growth and polarity in Gram-positive bacteria by localizing specifically to the cytoplasmic membrane at the cell poles and division septum, independent of other proteins. In this issue, Lenarcic *et al* suggest that DivIVA localization depends on the concave geometry of the membrane at those sites. This recognition of membrane curvature is reminiscent of another bacterial protein, SpoVM, as well as eukaryotic BAR-domain proteins.

Originally isolated as a regulator of cell-division site placement in *Bacillus subtilis*, DivIVA and its orthologues are found in a diverse array of Gram-positive bacteria (Edwards *et al*, 2000; Fadda *et al*, 2007). In *Streptomyces*, *Corynebacterium* and *Mycobacterium* species that grow by apical extension instead of sidewall extension, DivIVA is required for normal growth. It localizes to cell poles, cell-division septa and emerging branches, which are all areas of active growth (Fadda *et al*, 2007; Hempel *et al*, 2008; Kang *et al*, 2008; Letek *et al*, 2008). In cocci, such as *Streptococcus pneumoniae* that grow at their septum, DivIVA preferentially localizes there (Fadda *et al*, 2007).

Although DivIVA localizes similarly to the division septum and cell poles of the rod-shaped bacterium *Bacillus subtilis*, it is dispensable for cylindrical wall growth, which is orchestrated by the actin homologue MreB. Instead, DivIVA organizes cell polarity in this species. It initially localizes to the invaginating division septum after the cell-division protein FtsZ has assembled the Z ring. DivIVA then recruits MinJ and the MinCD complex, which inhibits aberrant assembly of additional Z rings at the septum and daughter cell poles, thus spatially restricting septal-wall growth to midcell (Bramkamp *et al*, 2008). In addition, at early stages of *B. subtilis* sporulation, polarly localized DivIVA interacts with RacA, which binds a centromere-like site on the chromosome. This interaction helps to pull the chromosome poleward into the pre-spore before the asymmetric septum closes (Bramkamp *et al*, 2008).

The above roles for DivIVA strongly suggest that its polar and septal localization is essential for its function (Figure 1). Importantly, this positioning of DivIVA itself is not dependent on any other known protein: when expressed in species normally lacking DivIVA, such as *Escherichia coli* or even the yeast *Schizosaccharomyces pombe*, it still localizes to the cell poles and septum (Edwards *et al*, 2000). This was the first hint that DivIVA localization might recognize specific membrane lipids, a physical cue, such as membrane curvature, or both.

This study by Lenarcic *et al* addresses this hypothesis. They first showed that DivIVA targets the membrane through a predicted N-terminal amphipathic helix. When fused to GFP and expressed in *B. subtilis* or *E. coli*, this helix was sufficient for binding anywhere on the membrane, but the rest of DivIVA was required for specific localization to cell poles or division septa. Using shape mutants of *E. coli*, the authors then showed that DivIVA–GFP localizes preferentially to regions of sharpest concavity, consistent with the curvature hypothesis. It is unlikely that DivIVA recognizes specific phospholipids that are enriched at the septum and cell poles of *B. subtilis*, because DivIVA was still able to localize to these regions in mutants deficient in these lipids.

These results prompted the authors to test the negative membrane-curvature hypothesis in greater detail. It is likely that multiple DivIVA molecules are involved in recognizing membrane curvature, because DivIVA oligomerizes into 'doggy-bone' structures *in vitro* (Stahlberg *et al*, 2004), probably through a conserved coiled-coil domain at its C terminus (Edwards *et al*, 2000; Lenarcic *et al*, 2009).

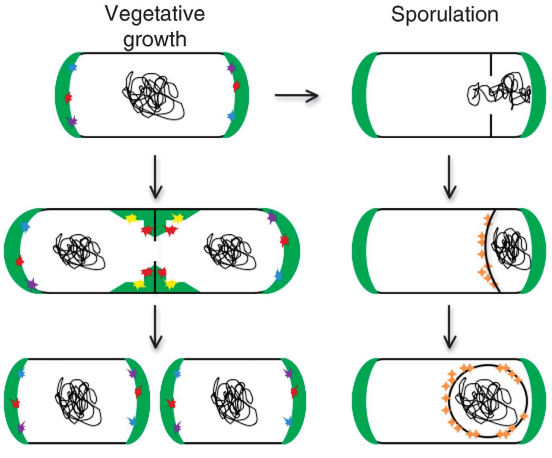

Figure 1 Roles of DivIVA and SpoVM in the *B. subtilis* life cycle. During vegetative growth, DivIVA oligomeric structures (green) localize to concave cell poles where they recruit other proteins (coloured blobs). When the cell begins to divide, the newly formed concave curvature attracts DivIVA to the septum. During sporulation, DivIVA–RacA pulls the chromosome inside the pre-spore before the septum closes. SpoVM (orange crosses) localizes to the outside of the increasingly convex spore membrane.

Moreover, the broad arcs of DivIVA localization around cell poles and division septa probably reflect a large membrane-bound lattice mediated by multiple oligomers. When DivIVA was added to purified liposomes, the protein not only bound strongly to the liposomes but also joined liposomes together, supporting the idea that DivIVA oligomerization on a membrane can attract additional DivIVA complexes. A mathematical simulation of membrane-associated DivIVA oligomers supported the idea that DivIVA binds to negatively curved membranes by protein–protein and protein–membrane bridging. One prediction is that inhibition of the coiled-coil interactions should abolish DivIVA localization.

This potential recognition of membrane geometry by DivIVA is reminiscent of *B. subtilis* SpoVM, a stand-alone, 26-residue amphipathic helix that recognizes curvature. However, SpoVM binds specifically to the positively curved (convex) membrane on the outside of the pre-spore during sporulation (Figure 1), and binds to the outside of lipid vesicles *in vivo* or *in vitro* only if they have sufficiently sharp positive curvature (Ramamurthi *et al*, 2009). It is not clear why DivIVA, if it indeed prefers concave shapes, can bind so efficiently to the outside of convex liposomes. One rationale is that the amphipathic helix of DivIVA may have a stronger general affinity for lipid membranes than SpoVM. Moreover, perhaps DivIVA prefers membranes with spherical shape, such as a cell pole, versus those with cylindrical shape, such as the cell sidewall.

The postulated targeting of DivIVA and SpoVM to distinct regions of curvature is roughly analogous to the membrane-targeting mechanisms of eukaryotic BAR-domain proteins (BARs). Like DivIVA, BARs contain conserved coiled-coil structures, and spatial specificity is determined by other variable protein domains (Frost *et al*, 2009). BARs induce membrane curvature *de novo* by triggering membrane deformation, which then triggers binding of additional BARs, generating a positive feedback loop of curvature proliferation. The apparent ability of DivIVA oligomers to initiate hyphal branches from straight sidewall in *Streptomyces* suggests that they also induce membrane curvature and, like BARs, reinforce their localization.

The concave curvature-mediated targeting of DivIVA proposed by Lenarcic *et al* suggests that other proteins that localize specifically to the division septum and/or cell poles may also use similar mechanisms. However, understanding the molecular mechanisms of DivIVA and SpoVM localization will require more details of the structures built by these proteins in conjunction with the membrane. The importance of DivIVA, in particular, for the proliferation of Gram-positive bacterial pathogens should place these studies at the leading edge.

Conflict of interest

The authors declare that they have no conflict of interest.

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